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Detection of ζ-Globin Chains in the Cord Blood by ELISA (Enzyme-Linked Immunosorbent Assay): Rapid Screening for α-Thalassemia 1 (Southeast Asian Type)

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Fetuses with homozygous α-thalassemia 1, in which the deletion of all four α-globin genes results in the absence of any α-globin chains, are severely anemic with clinical features of hydrops fetalis. Definitive diagnosis of α-thalassemia 1 carriers is difficult since there are few red cell abnormalities. Recently Chui et al. found that minute amounts of embryonic ζ-globin chains are present in adult hemoglobin of the Southeast Asian type of α-thalassemia 1 carriers.

In this study, we screened 521 cord bloods for α-thalassemia 1. Hemoglobin analysis, including quantitation of Hb Bart's, was performed using the automated HPLC, α-thalassemia short program (VARIANT, Bio-Rad, Hercules, CA). Of these, 200 cord blood samples in which Hb Bart's was demonstrated were tested for the presence of ζ-globin chains by ELISA using labeled anti-ζ monoclonal antibody. ζ-Globin ranged between 0.21 and 0.83% in 19 specimens carrying α-thalassemia 1 gene. In the remaining 90 out of 109 specimens in which Hb Bart's was greater than 1.2%, ζ-globin was less than 0.17%. DNA analysis revealed the presence of normal α-genotype and other types of α-thalassemia including α-thalassemia 2 and Hb Constant Spring. One false positive was found in which the ζ-globin was 0.25% by ELISA but in which PCR indicated an α-thalassemia 2 heterozygote. Ninety-one samples with Hb Bart's of less than 1.2% by HPLC are most likely normal with a ζ-globin range between 0 and 0.14%. This study also showed that the frequency of α-thalassemia 1 in Bangkok is 3.65%. Am. J. Hematol. 57:283–286, 1998.

Key words: zeta-globin antibody; ELISA; α-thalassemia 1

INTRODUCTION

During human embryonic and fetal development, an orderly change occurs in hemoglobin (Hb) production. The earliest embryonic Hbs are Hb Gower 1 ($\zeta_2 \epsilon_2$), Hb Gower 2 ($\alpha_2\epsilon_2$), and Hb Portland I ($\zeta_2 \gamma_2$). During the second and third trimesters of gestation, fetal Hb ($\alpha 2\, \gamma 2$) becomes the dominant Hb until shortly after birth, when it is gradually replaced by Hb A ($\alpha_2\beta_2$) [1].

ζ-Globin chains, the α-globin-like chains found in the embryo and fetus, are generally believed to be present only during the first 2 months of gestation [2]. However, when all four α-globin genes are deleted, as in the Hb Bart's hydrops fetalis syndrome, ζ-globin chains continue to be synthesized even in the third trimester of gestation. Recently, minute amounts of ζ-globin chains were shown to be present in hemolysates from adult individuals with α-thalassemia 1 due to the ($--^{SEA}$/) deletion [3]. A novel anti-human ζ-globin chain monoclonal

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antibody has been reported to detect α-thalassemia 1 in a slot blot immunobinding assay [4].

In general, the α-thalassemia trait is difficult to recognize. Individuals who have decreased red cell osmotic fragility, low MCV, and slightly abnormal red cell morphology with normal HbA_2 levels are suspected of being α-thalassemia 1 carriers. Neonatal diagnosis of α-thalassemia carriers may be possible by determination of the proportion of Hb Bart's in cord blood. Definitive diagnosis of α-thalassemia requires DNA analysis. In this study, a sensitive and specific enzyme-linked immunosorbent assay (ELISA) for human embryonic ζ-globin chains was used to detect α-thalassemia in newborn cord blood samples.

MATERIALS AND METHODS

Cord blood samples (521) were randomly collected by direct aspiration from the umbilical vessels at the delivery room of the Department of Obstetrics and Gynecology, Siriraj Hospital, Bangkok, Thailand. Hemolysates were prepared from packed red cells for Hb analysis and ELISA. Hemoglobin types and quantitation of Hb Bart's were performed by the automated HPLC, α-thalassemia short program (VARIANT, Bio-Rad, Hercules, CA).

Monoclonal Antibodies to ζ-Globin Chains

Monoclonal antibodies were generated in BalbC mice to purified ζ-globin chains. The antibodies did not react with hemoglobins A, S, F, C, and E. Antibodies were purified from ascites on Protein G columns. The purified antibodies were conjugated to horseradish peroxidase using a meta-periodate technique.

ELISA for ζ-Globin Chains

A standard curve consisting of 0, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8% ζ-globin, was prepared from normal blood hemolysate mixed with hemolysate from a Hb Bart's hydrops fetalis sample in which the amount of ζ-globin was determined by HPLC. A portion (10 μl) of each sample was diluted in 100 μl of 50 mM Tris HCl, 0.15 M NaCl, pH 7.5, and incubated at 37°C for 30 min to allow the ζ-globin in hemolysate to bind to the surface of the Costar (Cambridge, MA) microtiter plate. After rinsing with TS, 100 μl of a 1:4,000 dilution of 3E10-HRP conjugate (horseradish peroxidase conjugated anti-ζ antibody, ISOLAB Inc., Akron, Ohio) in 1% bovine serum albumin in TST (Tris-Saline-Tween 20) was added and allowed to react at 37°C for 60 min. After rinsing, 100 μl of the substrate TMB (3, 3′, 5, 5′,tetramethylbenzidine) was added, allowed to react for 5 min, and stopped by the addition of 100 μl of 0.2 M sulfuric acid. The absorbance at 450 nm was measured with an ELISA plate reader. The ζ content of the samples was calculated from the standard curve run with each batch of samples.

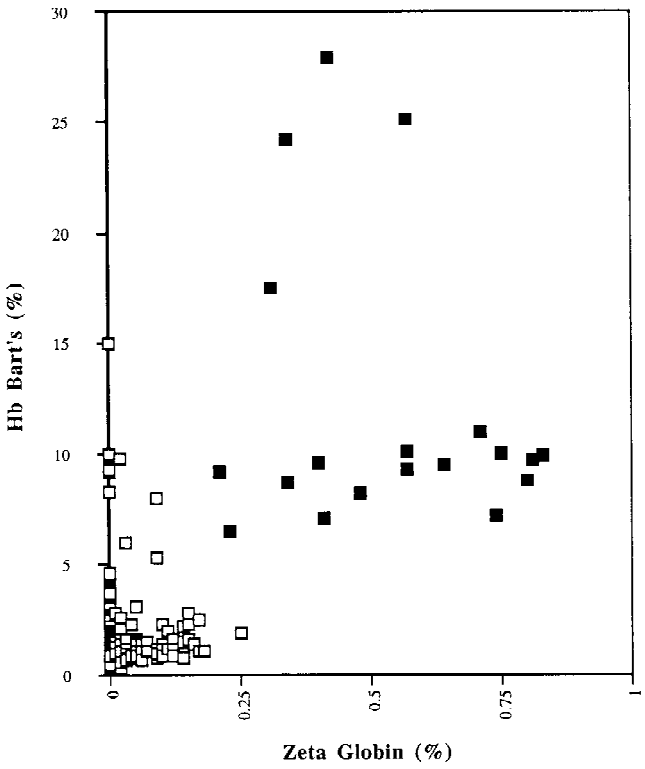

Fig. 1. Detection of α-thalassemia 1 by anti-ζ-globin antibody in 200 cord blood. With combination of the measurement of Hb Bart's by HPLC and ζ-globin by ELISA, the data appear to fall into four groups. (1) Hb Bart's <1.2% and ζ-globin <0.14%; normal (αα/αα); (2) Hb Bart's >1.2% and ζ-globin <0.17%; normal (αα/αα) or α-thalassemia 2 heterozygote (−α/αα) or α-thalassemia 2 homozygote (−α/−α) or Hb CS heterozygote ($\alpha^{CS}\alpha/\alpha\alpha$); (3) Hb Bart's 6–11% and ζ-globin 0.21–0.83%; α-thalassemia 1 heterozygote (−−/αα); (4) Hb Bart's >15% and ζ-globin 0.3–0.6%; Hb H disease (−−/−α or −−/$\alpha^{CS}\alpha$). ■, Individuals carrying an α-thalassemia-1 gene; □, no α-thalassemia-1 gene.

PCR techniques to determine the α-thalassemia 1 [5], α-thalassemia 2 (both $-\alpha^{3.7}$ and $-\alpha^{4.2}$ types) [6], and Hb Constant Spring [7] were carried out with DNA extracted from buffy coat cells.

RESULTS

Hb Bart's was detected in 200 out of 521 cord blood samples by the automated HPLC. Quantitation of ζ-globin chains was carried out by ELISA in the samples positive for Hb Bart's. Figure 1 shows the ζ-globin content and the level of Hb Bart's in each individual. Of these, 109 had levels of Hb Bart's greater than 1.2% as determined by HPLC. Table I shows the amounts of ζ-globin chains as determined by ELISA. Elevated amounts of ζ-globin were detected in 19 out of 200 specimens. PCR technique confirmed the presence of α-thalassemia 1 genotype in this group. There were two Hb

TABLE I. Amounts of ζ-Globin Chains Detected by ELISA Technique in 200 Samples With Positive Hb Bart's by the Automating HPLC

		ζ-Globin chains (%)	
	No.	Range	Mean ± SD
Normal	91	0–0.14	0.01 ± 0.03
α-Thalassemia 1	15	0.21–0.83	0.57 ± 0.21
Hb H Disease	2	0.34, 0.57	—
EA Bart's	1	0.42	—
EF Bart's	1	0.31	—
Other α-thalassemia	90	<0.17	0.04 ± 0.06

H disease specimens (α-thalassemia 1/α-thalassemia 2) that had ζ-globins 0.34 and 0.57% with Hb Bart's of 24.2 and 25.1%, respectively. There was one sample each with AE Bart's disease (α-thalassemia 1/α-thalassemia 2-EA) and EF Bart's disease (α-thalassemia 1/α-thalassemia 2-EE) in which ζ-globin was 0.42 and 0.31%, respectively, with Hb Bart's of 27.9 and 17.5%, respectively. The remaining 15 specimens were α-thalassemia 1 heterozygotes in which the ζ-globins ranged between 0.21 to 0.83% (mean ± SD = 0.57 ± 0.21%) whereas the range of Hb Bart's was 6 to 11%. This study also showed that the gene frequency of α-thalassemia 1 in Bangkok is 3.65%.

Ninety out of the 109 specimens in which Hb Bart's was greater than 1.2% had either normal α-globin or other types of α-thalassemia. All had ζ-globins less than 0.17% (mean ± SD = 0.04 ± 0.06%). Among these, three were double heterozygotes for α-thalassemia 2 and Hb Constant Spring (Hb Bart's was 9.2, 9.3, and 9.4%), one Hb Constant Spring homozygote (Hb Bart's was 15%), and 12 homozygotes for α-thalassemia 2 (Hb Bart's 3.9–10%). The remaining were either heterozygotes for α-thalassemia 2 (Hb Bart's was 1.8 ± 0.5%). Hb Constant Spring (Hb Bart's was 2.6 ± 0.47%), or normal (Hb Bart's was 0.7 ± 0.37%) (Fig. 1). Those in which Hb Bart's was less than 1.2% (91 cases) most likely had the normal phenotype. In these cases ζ-globins ranged from 0 to 0.14% (mean ± SD = 0.01 ± 0.03%). One false-positive sample with Hb Bart's 1.9% and a ζ-globin of 0.25% by ELISA was found, which PCR indicated was an α-thalassemia 2 heterozygote.

DISCUSSION

Infants with hemoglobin Bart's hydrops fetalis syndrome (homozygous α-thalassemia 1), with the deletion of all four α-globin genes, are stillborn or die shortly after birth. Pregnancies involving Hb Bart's hydrops fetalis syndrome are associated with an increased risk of maternal complications, such as hydramnios, pre-eclampsia, antepartum or post-partum hemorrhage, and difficult vaginal delivery [8,9]. There is also considerable emotional strain for the mothers and their immediate family members. Prenatal diagnosis for Hb Bart's hydrops fetalis syndrome is possible by means of Southern blotting or polymerase chain reaction by using DNA extracted from fetal cell samples, obtained by either chorionic villi biopsy or amniocentesis [10–13]. However, the identification of couples at risk of conceiving fetuses afflicted with Hb Bart's hydrops fetalis syndrome is problematic, and is presently dependent on the history of birth of a previous hydropic fetus.

Recently, Chui et al. and Luo et al. have found that minute amounts of embryonic ζ-globin chains were present in adult carriers of the Southeast Asian type of α-thalassemia 1 [3,4]. In the current study, we have demonstrated that a much simpler enzyme-linked immunosorbent assay (ELISA) using the horseradish peroxidase conjugated anti-ζ antibody (3E10-HRP), can similarly detect ζ-globin chains, and can be used as a diagnostic test to detect carriers of α-thalassemia 1 due to the ($--^{SEA}/$) deletion. Previously we succeeded in using this approach to detect ζ-globin chains in specimens from 44 individuals carrying the α-thalassemia 1 gene, whereas none of the 80 normal individuals had detectable ζ-globin chains [14]. The data obtained in this study, with a combination of the measurement of Hb Bart's by HPLC and ζ-globin by ELISA, appear to fall into four groups: (1) Hb Bart's <1.2% and ζ-globin <0.14% (mean ± SD = 0.01 ± 0.03%); normal (αα/αα); (2) Hb Bart's >1.2% and ζ-globin <0.17% (mean ± SD = 0.04 ± 0.06%); normal (αα/αα) or α-thalassemia 2 heterozygote (−α/αα) or α-thalassemia 2 homozygote (−α/−α) or Hb CS heterozygote ($\alpha^{CS}\alpha/\alpha\alpha$); (3) Hb Bart's 6–11% and ζ-globin 0.21–0.83% (mean ± SD = 0.57 ± 0.21%); α-thalassemia 1 heterozygote (−−/αα); (4) Hb Bart's >15% and ζ-globin 0.3–0.6%; Hb H disease (−−/−α or $--/\alpha^{CS}\alpha$). The levels of ζ-globin in each group of subjects at different gestational ages of pregnancy were also assessed but they did not seem to be related to prematurity (data not shown).

The gene frequency of the ($--^{SEA}/$) deletion in Southeast Asia and Southern China is approximately 3% [8,15]. The current study has shown that the frequency of α-thalassemia 1 in Bangkok is 3.65%, which is in agreement with that previously reported [15]. Homozygous α-thalassemia 1 containing the ($--^{SEA}/$) deletion is the major cause of hydrops fetalis syndrome in this part of the world. The World Health Organization estimated in 1983 that there were probably 20,000 infants born annually afflicted with homozygous α-thalassemia 1 [16]. The simple ELISA described here is suitable for screening large populations for carriers of the ($--^{SEA}/$) deletion. The sensitivity and specificity of this test was 100 and 99.5%, respectively (Table II). The general application of this simple screening test for carriers of α-thalassemia 1 with the ($--^{SEA}/$) deletion in Southeast Asia and

TABLE II. Sensitivity and Specificity of ELISA Technique in the Detection of ζ-Globin Chains in Individual Cord Blood Containing α-Thalassemia 1*

	Samples with α-thal 1 (−−SEA)	Samples without α-thal 1 (−−SEA)	Total
Negative	0	180	180
Positive	19	1	20
Total	19	181	200

*Sensitivity $= \frac{19}{19} \times 100 = 100\%$; Specificity $= \frac{180}{181} \times 100 = 99.4\%$.

Southern China may help to improve the genetic counseling and the quality of obstetrical care provided to those women at risk of conceiving infants with Hb Bart's hydrops fetalis syndrome.

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